STUDY OF THE ELECTROPHORETIC FRACTIONS OF PROTEINS OF THE CARDIAC MUSCLE DURING THE DEVELOPMENT OF ATHEROSCLEROSIS

(UDC 616.13-004.6-092-07 : 616.127-008.939.6)

D. G. Grigor'yan and V. A. Shalimov

Laboratory of Biochemistry (Head—Docent V. A. Shalimov), Experimental Division (Head—Professor F. D. Vasil'enko) of the Central Institute of Spa Therapy and Physiotherapy (Director—Candidate of Medical Sciences, G. N. Pospelova), Moscow
(Presented by Member of the Academy of Medical Sciences, USSR, N. N. Zhukov-Verezhnikov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 59, No. 6, pp. 56-58, June, 1965
Original article submitted March 20, 1964

Our earlier investigations showed that in pronounced atherosclerosis, the content of protein fractions of the cardiac muscle is substantially changed [1].

In this work we decided to determine how the appearance and development of the changes that we detected are correlated in time with the process of development of experimental atherosclerosis.

EXPERIMENTAL PROCEDURE

The experiments were conducted on 42 rabbits. Experimental atherosclerosis was reproduced in 34 animals according to N. N. Anichkov's method by daily feeding cholesterol in a dose of 0.2 g per kilogram of weight.

The cardiac muscle proteins of the animals were investigated on the 10th, 20th, 30th, 60th, 100th, and 120-130th days of development of atherosclerosis. Five to six animals were examined at each stage of development of the pathological process. The control group comprised eight healthy rabbits.

The total blood serum cholesterol was determined according to the Éngel'gardt-Smirnova method.

The animals were killed by air embolism. The degree of atherosclerotic change in the aorta was studied macroscopically according to a four-point system. The cardiac muscle (left ventricle) was pulverized on a freezing microtome. The proteins were extracted with phosphate buffer pH 7.45 at a temperature from 0 to 2°, then centrifuged for 15 min at 4000 rpm in the cold.

The electrophoretic separation of the proteins of the extracts was conducted in phosphate buffer, pH 6.4, with an ionic strength of 0.058 [3, 4, 5] for 18-19 h at a voltage of 250. The strips were colored with acid blue-black dye.

The relative content of the protein fractions was determined photometrically on the FÉK-M instrument.

RESULTS OF THE EXPERIMENTS

Data on the ratio of the cardiac muscle protein fractions in the normal state and during the reproduction of atherosclerosis (10-130 days) are presented in the Table.

Three groups of fractions, corresponding to the protein muscle components in extracts of rabbit muscles *l*, m, n (myogen), K_1, K_2 (phosphorylase), and myoalbumin [3, 4, 5] were detected on the electrophoretograms of the protein extracts.*

*The phoretograms obtained were cited in our previous report [1].

Ratio of Cardiac Muscle Protein Fractions of Rabbits at Various Stages of Development of Experimental Atherosclerosis (in % of the sum of the fractions, taken as 100)

Duration of feeding of cholesterol (in days)	No. of animals	Cholesterol content in blood serum (in mg%)	Fractions							
			Myoalbumin	Proteins possessing phosphorylase activity			n	m	l	Sum n+m+l
				K_2	K_1	Sum $K_2 + K_1$				
Norm	8	40,0	20,40±0,8	12,68±0,9	24,00±1,7	36,68±1,4	26,97±0,4	10,53±0,7	5,31±0,8	42,91±0,8
10	6	171,0	20,07±0,5	15,16±0,6	36,09±1,6	51,25±2,5	14,63±0,4	12,29±2,7	—	28,66±1,6
20	6	131,0	21,48±0,5	17,00±0,7	20,52±0,8	37,52±0,6	16,41±0,4	12,51±0,2	12,06±1,1	40,98±0,9
30	5	200,0	20,04±1,0	18,36±0,8	22,64±0,9	41,00±1,4	26,62±0,8	11,54±0,9	—	29,01±1,4
60	6	435,0	23,92±1,3	16,23±1,1	20,11±1,3	36,34±1,1	26,50±0,5	11,08±0,3	—	39,62±1,5
100	5	709,0	23,76±1,0	13,69±1,1	24,55±1,6	38,24±0,5	22,84±1,7	12,22±1,0	—	37,98±1,2
120—130	6	900,0	20,16±0,9	15,52±1,4	28,53±1,4	44,10±1,9	25,23±1,5	9,89±0,5	—	35,84±1,1

Note: Average values obtained in an investigation of the cardiac muscle proteins of five to six animals and the average error of the arithmetic means are cited; macroscopic injury to the aorta was observed in the rabbits on the 100th (++) and 130th (+++) days of reproduction of atherosclerosis.

Depending on the period of development of atherosclerosis, various ratios of the cardiac muscle protein fractions were observed.

The greatest changes were noted in the fraction of proteins possessing phosphorylase activity ($K_2 + K_1$) and in the fraction of myogens (*l*, m, n). These changes were the most pronounced during the earliest and latest stages of development of the pathological state. Thus, on the tenth day of feeding the animals with cholesterol, the fraction of proteins possessing phosphorylase activity ($K_2 + K_1$) was substantially increased (from 36.68 to 51.25%). Beginning with the 20th day of development of the pathological process, the content of the fraction $K_2 + K_1$ differed little from the norm, and only on the 120th-130th days of development of atherosclerosis did it again increase, reaching 44.1%.

The content of the myogen fraction also underwent changes during various periods of feeding cholesterol to the animals. Moreover, the greatest changes also pertained to the tenth and 120-130th days of development of pathology. The average content of the myogen fraction was substantially reduced on the tenth day, comprising 28.66%, while the content of the myogen fraction of the cardiac muscles of healthy animals corresponded to 42.91%.

However, from the 12th to the 100th days of development of atherosclerosis, a less appreciable decrease in the content of this fraction was observed in comparison with the norm. In addition, a certain redistribution of the proteins of the myogen fraction was detected on the 20th day.

On the 120-130th days of feeding of the animals with cholesterol, the average content of the myogen fraction was again reduced, comprising 35.84%.

In an analysis of the data on the myoalbumin content in the extracts of rabbit cardiac muscles, we found no significant changes during the entire period of development of experimental atherosclerosis. A negligible increase in the average content of the myoalbumin fraction was observed on the 60th and 100th days of development of pathology.

The results of our investigations coincide with the literature data [2], indicating different degrees of change of the protein metabolism of the organs and tissues depending on the period of development of atherosclerosis.

Thus, on the basis of our experimental work, we may conclude that the greatest change in the protein metabolism of the cardiac muscle occurs at the earliest and latest stages of development of atherosclerosis.

LITERATURE CITED

1. D. G. Grigor'yan and V. A. Shalimov, Byull. Éksper. Biol., No. 2, (1964), p. 61.
2. M. G. Kritsman and M. V. Bavina, In the book:

Atherosclerosis and Coronary Insufficiency [in Russian], Moscow, (1956), p. 126.
3. F. Z. Meerson, Compensatory Hyperfunction and Cardiac Insufficiency [in Russian], (1960).
4. A. Mariani and G. Toschi, Atti Accad. nazl. Lincei, Rend., Classe Sci. fis., mat. e nat., 14, (1953), p. 285.
5. G. Toschi and A. Mariani, Ibid., 16, (1954), p. 365.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
